

**Metabolically inert perfluorinated fatty acids directly activate uncoupling protein 1 in brown-fat mitochondria**

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**Online Resource 3**

**Parameters of Michaelis-Menten kinetics fitting of concentration-response curves of PFOA, PFOS and octanoic acid in brown-fat mitochondria**

| Parameters   | UCP1-dependence                  | PFOA          | PFOS          | Octanoic acid   |
|--|----------------------------------|---------------|---------------|-----------------|
| $K_m$<br>$\mu\text{M}$   | UCP1-dependent<br>(WT – UCP1 KO) | 162           | N/C           | 399             |
|  | UCP1-independent<br>(UCP1 KO)    | $676 \pm 110$ | $243 \pm 162$ | $2503 \pm 12\#$ |
| $V_{\max}$<br>$\frac{\text{nmol O}_2}{\text{min} \cdot \text{mg}}$ | UCP1-dependent<br>(WT – UCP1 KO) | 51            | N/C           | 32              |
|  | UCP1-independent<br>(UCP1 KO)    | $49 \pm 10$   | $24 \pm 6$    | $92 \pm 7\#$    |

UCP1-independent  $K_m$  and  $V_{\max}$  are mean  $\pm$  S.E. obtained from analysis of each individual concentration-response curve for PFOA, PFOS, and octanoic acid for the best fit option of the GraphPad Prism application for adherence to simple Michaelis-Menten kinetics; n = 2-3.

UCP1-dependent  $K_m$  and  $V_{\max}$  are necessarily based on the subtracted mean values only but are thus indirectly based on 5-6 independent preparations.

N/C: not calculable (concentration-response curve for PFOS does not fit to simple Michaelis-Menten kinetics)

# indicates statistically significant difference between octanoic acid and PFOA ( $P < 0.05$ )